L-theanine partially counteracts caffeine-induced sleep disturbances in rats

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A B S T R A C T

L-theanine has been reported to inhibit the excitatory effects of caffeine. The present study examined the effects of L-theanine on caffeine-induced sleep disturbances in rats. Rats received the following drug pairings: saline and saline (Control), 7.5 mg/kg caffeine and saline, or 7.5 mg/kg of caffeine followed by various doses of L-theanine (22.5, 37.5, 75, or 150 mg/kg). Vigilance states were divided into: wakefulness (W), transition to slow-wave sleep (SWS), slow-wave sleep (SWS), and rapid-eye-movement sleep (REMS). Caffeine significantly increased the duration of W and decreased the duration of SWS and REMS compared to the Control. Although L-theanine failed to reverse the caffeine-induced W increase, at 22.5 and 37.5 mg/kg (but not at 75 and 150 mg/kg), it significantly reversed caffeine-induced decreases in SWS. In conclusion, low doses of L-theanine can partially reverse caffeine-induced reductions in SWS; however, effects of L-theanine on caffeine-induced insomnia do not appear to increase dose-dependently.

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1. Introduction

Caffeine is one of the most widely used psychoactive substances, best known for its effects as a behavioral stimulant. It is thought to exert its central nervous system effects primarily through adenosine receptor blockade (Deckert and Gleiter, 1989). Caffeine produces a variety of sleep disturbances, including reduction of total sleep time, prolonged latency of sleep onset, and increased wakefulness in humans and rats (Bonnet and Arand, 1992; Karacan et al., 1976; Paterson et al., 2007; Schwierin et al., 1996; Shinomiya et al., 2004).

In addition, caffeine administration has been used as a model of insomnia in rats (Bonnet and Arand, 1992; Karacan et al., 1976; Paterson et al., 2007). Previous studies have demonstrated that caffeine intake is a common cause of sleep disruption, and that reducing caffeine intake increases sleep quality (Edelstein et al., 1984; Morgan et al., 1989; Walsh et al., 1986).

Caffeine is usually ingested by drinking coffee or tea. The caffeine contents of coffee and tea in 200 ml serving of instant coffee and same volume of brewed tea are about 80 mg and 40 mg, respectively (Barone and Roberts, 1996). A recent report states that, adults in the UK consume about 240 mg of caffeine a day, mainly from drinking coffee or tea. The caffeine values were: instant coffee 54 mg, ground coffee 105 mg and tea 40 mg per serving (Heatherley et al., 2006). Although coffee and tea provide doses of caffeine sufficient to induce a marked alertness and psychomotor activation, it is commonly considered that drinking tea is less stimulating and more relaxing than drinking coffee. This effect is thought to be due to L-theanine, an amino acid analog present in tea but not in coffee. A cup of tea is estimated to contain 20–50 mg of L-theanine (Rogers et al., 2008). L-theanine has various pharmacological actions such as promoting feelings of calmness, decreasing alertness, and anti-stress effects (Haskell et al., 2008; Kimura et al., 2007). Additionally, Kakuda et al. (2000) showed that by measuring electroencephalography (EEG) in rats intravenous L-theanine at a dose higher than 0.78 mg/kg, could inhibit the stimulatory action of caffeine.

Here, we propose that L-theanine counteracts the effects of caffeine on sleep. Therefore, this study aimed to evaluate the effects of L-theanine on sleep/wake architecture during caffeine-induced sleep-wake disturbance in rats, and to determine whether L-theanine could reverse the sleep-disrupting effects of caffeine.

2. Materials and methods

2.1. Animals

Eight adult male Sprague–Dawley rats (Samtaco, Osan, Korea) were used, weighing 260–320 g at the time of surgery. Animals were individually housed in stainless steel cages (20 cm × 35 cm × 17 cm) throughout...
the study. They were maintained in a controlled environment for the duration of the study: 21–24 °C ambient temperature, 12:12 light–dark cycle (lights on from 7:00 to 19:00), with food (Hyochang Science, Daegu, Korea) and tap water available ad libitum except on the day of the experiment. The experiments were approved by the Kyungpook National University Institutional Animal Care and Use Committee, and were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1996). The rats were euthanized by CO2 overdose after the completion of the experiment.

2.2. Drugs and materials

L-theanine (Sigma-Aldrich Inc., St. Louis, MO, USA) and caffeine (Junsei Chemical Co., Tokyo, Japan) were dissolved in distilled water. The concentration of each drug solution was adjusted so that the volume injected was constant at 1.0 ml/kg body weight. All drugs were prepared daily, and were administered intraperitoneally (i.p.). Previous studies showed that caffeine-induced insomnia was dose-dependent with 7.5 mg/kg i.p. caffeine maintaining wakefulness for at least 2 h (Kwon et al., 2006).

2.3. Procedures

Rats were anesthetized (0.4 mg/kg of medetomidine and 60 mg/kg of ketamine, i.p.), and EEG and EMG recording electrodes were implanted. Once the animals showed no response to tail pinch, they were placed in a stereotaxic apparatus, and a mixture of 2% lidocaine and epinephrine was injected subcutaneously into the scalp to provide local anesthesia and reduce bleeding. An incision was then made in the midline of the scalp, and the skull surface was cleaned. Four small holes were drilled bilaterally into the parietal bones (5.0 mm posterior to and 2.5 mm lateral from bregma) and the interparietal bones (10.0 mm posterior to and 1.25 mm lateral from bregma) using a low-speed burr (ball diameter 0.8 mm) without perforating the dura mater. Gold-plated stainless steel screws (tip diameter 1.1 mm) were screwed tightly into the burr holes. Two screws over the cerebellum (in the interparietal bones) served as reference and ground electrodes. Pins were directly soldered to EMG wires and were connected to screw electrodes using enamel-coated copper wire, and then free-end of pins were arranged to connector in 3 × 2 matrices and fixed over the skull with dental acrylic. Systemic antibiotics (Cephradine inj., Korea Schnell Pharma Co., Ltd., Gyeonggido, Korea) and analgesics (Butophan inj., Myungmoon Pharmaceutical Co., Ltd., Seoul, Korea) were administered for 2 days, including the day of surgery, to prevent infection and minimize pain during recovery.

After at least one week of recovery, the rats habituated to the experimental procedure at least 2 times before recording. First, the rats were put in the recording box without any treatment from 10:30 to 17:30. Second, the rats were connected to the recording cable without drug treatment. After at least 7 days washout period, the rats were again connected to the recording cable one day before recording. The animals were connected to the recording equipment using a swivel and flexible tether cable system that allowed free movement in the chamber.

On the day of the experiment, the rats were put into the recording chambers at 10:00. After 3.5 h of habituation, the rats received drug injections at 13:20 and 13:30 according to the predetermined treatment plan; saline followed by saline ('Control' group), caffeine (7.5 mg/kg) followed by saline ('CT0' group), or caffeine (7.5 mg/kg) followed by different doses of L-theanine (22.5, 37.5, 75 and 150 mg/kg; groups 'CT1', 'CT2', 'CT3', and 'CT4', respectively). Electrophysiological activity was then recorded from 13:30 to 17:30. Each rat had at least one week washout period between sessions.

2.4. Recording

Two-channel EEG signals over the parietal cortex were measured in monopolar fashion with respect to the reference electrode. EEG signals were amplified by a factor of 10,000 (Model 3500, A-M Systems, Inc., Carlborg, WA, USA) and bandpass filtered (1 to 500 Hz). A 60-Hz notch filter was also used to remove electric hum. EMG signals were filtered between 1 and 500 Hz and amplified by a factor of 10,000. Analog signals were sampled by an AD converter (DAQ Pad6015, National Instruments Inc., Union City, CA, USA) using the LabView program (National Instruments, Inc., Union City, CA, USA) and digitized at 1 KHz, averaged every five consecutive samples and saved 200 Hz samplings to a disk.

2.5. Evaluation and data analysis

Sleep–wake state was scored manually using 10-s epochs of EEG and EMG activity. The scorer was blind to the substances used. Each

![Fig. 1](image-url). Representative hypnograms during 4 h after saline (Cont), caffeine (7.5 mg/kg, CT0), caffeine with L-theanine (37.5 mg/kg, CT2) administration. Post-treatment recording was established from 13:30 to 17:30. W, wakefulness; tSWS, transition to SWS; SWS, slow-wave sleep; and REMS, rapid-eye-movement sleep.
vigilance state [wakefulness (W), transition to slow wave sleep (tSWS), slow wave sleep (SWS), and rapid-eye-movement sleep (REMS)] was determined according to the following criteria: W, EEG theta rhythm and/or low-amplitude fast activity with phasic EMG signals; tSWS, EEG delta waves occupying less than 50% of the epoch and/or sleep spindles, with reduced EMG tone compared to W; SWS, slower and large amplitude delta waves, occupying more than 50% of the epoch with reduced EMG tone; and REMS, continuous EEG theta activity and/or large amplitude spindle activity with minimal EMG activity.

For each state, the following parameters were evaluated: 1) state duration, i.e., total time spent in each state over the entire recording period; 2) number of episodes per hour of each state, i.e., total number of episodes of each state per hour; and 3) mean episode duration, i.e., average duration of each episode of a particular state.

In order to quantify the effect of L-theanine on caffeine-induced insomnia, the latency to 1 h of cumulative sleep was measured as the amount of time from drug treatments to the accumulation of 360 10-s epochs (i.e., 1 h) scored as sleep, as described in the literature (Wisor et al., 2005).

2.6. Statistical analysis

All data are expressed as mean±S.E.M. Statistical comparisons of each sleep–wake state between groups were performed using one-way ANOVA and followed by Bonferroni post hoc testing (SPSS 12.0K; DataSolution, Seoul, Korea). P-values <0.05 were considered to be statistically significant.

3. Results

We found that caffeine (CT0) induced sleep disturbances which were indicated by: the increase of total duration and mean episode duration of W, the decrease of total duration and number of episode of SWS and REMS, and the increase of sleep latency, while L-theanine partially counteracted caffeine-induced decrease of SWS duration significantly. They were also noted in representative hypnograms after saline (Control), caffeine (CT0) and caffeine with L-theanine 37.5 mg/kg (CT2) in one animal (Fig. 1).

3.1. Duration during total recording time (4 h)

Duration at W was significantly enhanced by caffeine administration in all experimental groups (p<0.01) when compared to Control group (Fig. 2). SWS and REMS were significantly reduced by caffeine treatment in all groups (p<0.01) when compared to Control group, but the time spent at SWS in CT1 and CT2 group were significantly reversed (p<0.05) when compared to CT0 group.

3.2. Number of episodes per hour during total recording time (4 h)

Caffeine and all doses of L-theanine did not significantly affect W and tSWS occurrence when compared to Control group (Fig. 3). Caffeine and all doses of L-theanine significantly reduced the number of episodes at SWS and REMS (p<0.05 or p<0.01) when compared to Control, except at SWS in CT4 group.

3.3. Mean episode duration during total recording time (4 h)

CT0, CT2 and CT3 group only at W showed significantly increased mean episode duration (p<0.05) when compared to Control, except at SWS in CT4 group.

3.4. The latency to 1 h of sleep

A one-way ANOVA and Bonferroni test showed that caffeine and all L-theanine treatment groups significantly delayed the latency of 1 h of sleep (p<0.01) (Fig. 5). CT1 group tended to show shorter latency when compared to CT0 group (p=0.054) (Fig. 5).
4. Discussion

The present results show that L-theanine administration could reverse the caffeine-induced reduction in SWS in rats, although it failed to affect the W enhancement and REMS reduction produced by caffeine.

In the present study, the SWS-enhancing effect of L-theanine did not increase dose-dependently. L-Theanine administered at 22.5 and 37.5 mg/kg following caffeine treatment significantly reversed the caffeine-induced reduction in SWS, whereas L-theanine given at 75.0 and 150.0 mg/kg did not. The actions of L-theanine on W duration appeared to correspond to an inverted U-shaped dose–response curve; however, this effect was not statistically significant. These results were different from the previous study showing that effective concentration of L-theanine at 5 μmol/kg was needed to inhibit caffeine-induced brain wave excitation (Kakuda et al., 2000). This study suggests that L-theanine could block the effect of caffeine in a dose-dependent manner. Various factors may explain these discrepancies. First, they used the minimum dose of caffeine (0.97 mg/kg, i.v.) as a stimulant, while relatively larger dose of caffeine (7.5 mg/kg...
kg, i.p.) was used in this study. The higher dose of caffeine produced strong stimulatory action. Also, because doses of L-theanine used were different in each of the studies, different effects would be revealed under higher dose of L-theanine in the present study. Second, the evaluation method was different in both experiments, that is, our study estimated sleep-wake states but not EEG frequency power, whereas the study of Kakuda et al. (2000) estimated EEG frequency powers and showed increased delta and decreased beta activity. Distribution of increased delta wave within epoch was important to estimate slow-wave sleep, but decrease of beta activity always did not correspond to the increase of SWS duration. Third, because effect of L-theanine on sleep-wake architecture in the presence of caffeine did not increase dose-dependently, the attenuation effect of L-theanine may not be due to action at adenosine receptor level. Fourth, plasma concentration of both drugs may change due to time of administration. Finally, as L-theanine alone (0.348 mg/kg IV) induces an excitatory effect (Kakuda et al., 2000), excessive L-theanine dose in the case of CT3 and CT4 group exert its stimulatory action.

The data we present here have implications for the treatment of insomnia. In this study, we used caffeine administration as a model of insomnia in rodents, a technique previously validated by other groups (Bonnet and Arand, 1992; Karacan et al., 1976; Paterson et al., 2007). However, caffeine intake is a common cause of sleep disruption in humans, known to cause varying degrees of insomnia, and that the reduction in caffeine intake increases sleep quality (Edelstein et al., 1984; Morgan et al., 1989; Walsh et al., 1986). Thus, the effects of L-theanine we report here may be generalized to human populations suffering from insomnia due to caffeine consumption.

Our data revealed that low doses of L-theanine (22.5 and 37.5 mg/kg) following caffeine injection was able to reverse caffeine-induced sleep reduction. The effects of L-theanine on W were more prominent on mean episode duration while those on SWS were more prominent on the total number of episodes. Although none of the doses of L-theanine used reduced the latency to 1 h of sleep, the lowest dose produced a trend towards reduced latency, though this did not achieve statistical significance ($p = 0.054$).

In conclusion, L-theanine can attenuate the caffeine-induced reduction of SWS in rats, but this effect is not dose-dependent. As such, lower doses of L-theanine may help promote SWS in insomnia patients; however, excessive L-theanine intake may have the opposite effect and worsen sleep quality.

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References

Heatherley SJ, Mullings E, Tidbury M, Rogers P. Caffeine consumption among a sample of UK adults. Appetite 2006;47:266.